FORM REGULATION IN CERIANTHUS ÆSTUARII.

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During 1905–6 it was my privilege to enjoy for several months the facilities afforded by the laboratory of the San Diego Marine Biological Association at La Jolla, California. During this time the work which forms the subject of the present paper, together with other work to appear later was accomplished. I take this opportunity of expressing my appreciation of the kindness of Professor W. E. Ritter and the other members of the association in granting me the privileges of the laboratory.

In 1902–3 I made a study of the process of form-regulation in *Cerianthus solitarius* at Naples, the results of which have already appeared (Child, '03*a*–'05*b*). In order to test and compare the results obtained with that species an extensive series of experiments was performed with *Cerianthus æstuarii* (Torrey) which occurs in abundance on the tide-flats of Mission Bay near La Jolla. For the name of the species I am indebted to Professor H. B. Torrey, as his work on the species has not yet appeared.

As regards size and general appearance *C. æstuarii* does not differ widely from *C. solitarius*. It is, however, more delicate in structure, the body-wall and especially the muscular layer being much thinner than in *C. solitarius*. In consequence of this characteristic specimens which are all well distended with water often appear more or less translucent. The length of the animal varies greatly according to the degree of distension. Fig. 1 will serve to give an idea of the usual shape.

In general the results of my experiments with this species confirm in all essentials those obtained with *C. solitarius*, so that an extended account of most of the experiments is unnecessary. But the results of experiment differ in some respects in the two species, and these differences, being due largely to the differences in the character of the tissues, possess a certain interest.

C. æstuarii, like *C. solitarius*, inhabits burrows whose walls consist of slime, nematocysts and fine sand or mud. Like *C. soli-*

tarius also it is capable of living for months in clear water in the laboratory, though, as will appear below, certain interesting modifications of shape and proportions appear under these conditions. The description of structure and habits given for *C. solitarius*

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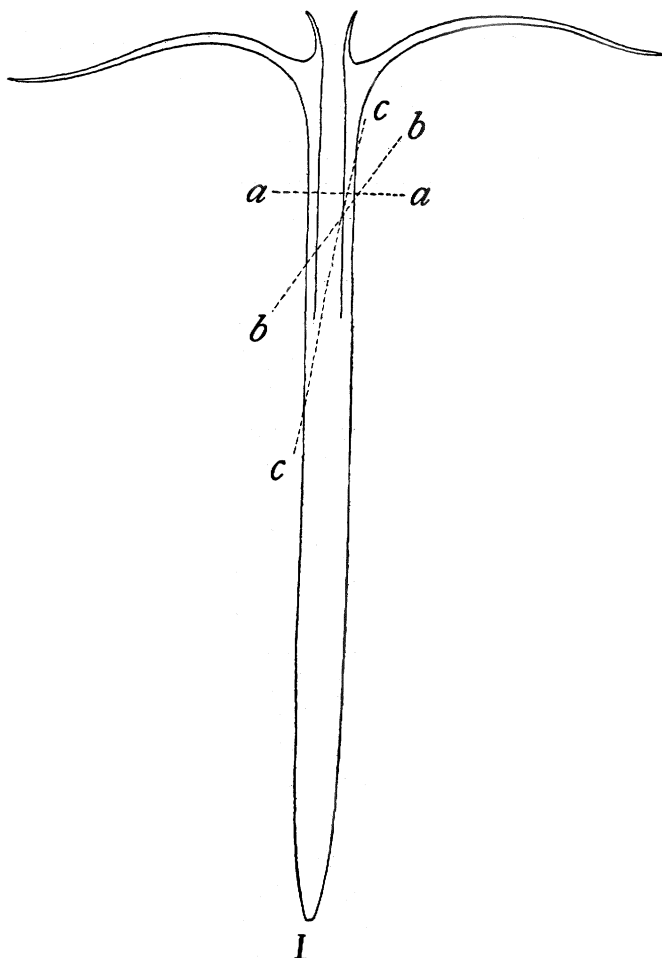
(Child, '03*a*, p. 239 et seq.) will apply in general to this species, but it may be noted here that *C. æstuarii* is more sensitive to external stimuli than *C. solitarius.* Slight contact-stimuli with needles or brush produce extreme and rapid contraction when the animal is normally distended with water, though it is apparently much less sensitive when partially or wholly collapsed.

Specimens normally distended are also very sensitive to light : if kept in covered dishes sudden exposure even to diffuse daylight produces marked general contraction after a short latent period. As regards this stimulus also collapsed specimens or pieces are much less sensitive than those which are distended with water.

The distension of the body and the erection of the tentacles is accomplished in this species as in *C. solitarius* by the entrance of water into the enteric cavity, both through the mouth and through the body-wall. The entrance of water through the body-wall probably occurs at all times to a greater or less extent, but is most readily observed in pieces undergoing regulation in which the ends have closed but no mouth has formed (cf. Child, '04*b*, p. 267 et seq.).

I. THE COURSE OF FORM-REGULATION.

In pieces above a certain minimal size, isolated by transverse cuts, the course of collapse and inrolling of the body-wall in the region of section, the closure of the wound and the formation of new tissue are in general similar to those processes in *C. solitarius* (Child, '03*a*, pp. 244–257, Figs. 1–24). As regards the formation of new tissue at the aboral end there is a quantitative difference between the two species, the amount of new tissue formed in *C. æstuarii* being usually much less than in *C. solitarius* (Child, '03*a*, pp. 257–259, Figs. 25–31). Attention may be recalled to the fact that the marginal tentacles in *C. solitarius* do not arise at the cut surface itself, but a short distance aboral to it (Child, '03*a*, p. 252, Figs. 10–19), the first indications of their formation being a reduction in the thickness of the body-wall in this region and the formation of a crenated ridge around the end of the piece, followed by the outgrowth of a tentacle above each interseptal chamber. These processes follow a similar course in *C. æstuarii*.

One difference between the two species may, however, be noted in this connection : in *C. æstuarii* the inrolling of the body-wall is much less regular than in *C. solitarius*, and closure in the usual manner is retarded or prevented more often in the latter species because the formation of the thin membrane closing the cut end is impossible. In consequence of the lack of firmness and stiffness in the body-wall of *C. æstuarii* the pieces often assume very

irregular forms when they collapse at the time of operation. Certain parts of the margin at the cut end may roll inward to a much greater extent than others, so that the cut surfaces on the two sides of the body may fail to approximate at all and closure cannot occur. The results of experiment are therefore less constant and uniform than in *C. solitarius*, though in general similar.

As regards the inrolling of cut margins in pieces of various form, longitudinal and transverse strips, oblique pieces, etc., all that has been said regarding *C. solitarius* (Child, '04*a*) will hold for this species. Here, in fact, results may vary even more widely than in *C. solitarius* because the body-wall is much more flaccid in this species after collapse. It is possible to inhibit completely the process of restitution simply by cutting the pieces of certain shapes so that the inrolling of the body-wall will prevent approximation of the cut margins : under these conditions closure of the opening and formation of the missing parts does not occur.

The regional differences in the power of regulation are very similar in the two species (cf. Child, '03*b*). The rapidity of oral form-regulation decreases with increasing distance of the level of section from the oral end.

As regards the relations between size of the piece and regulation the same similarity between the two species obtains. The length of tentacles is distinctly not proportional to the size of the piece (cf. Child, '03*b*, Part II., pp. 3–6). In *C. æstuarii* the uncertainty as to the size of minimal pieces is still greater than in *C. solitarius*, because of the great irregularity in the process of inrolling in short pieces.

In neither species has the outgrowth of new tissue directly from a free cut surface ever been observed (Child, '04*a*, pp. 66–74, Figs. 25–31 ; '04*b*, pp. 276–279, Figs. 3 and 4). On a free cut surface of the body-wall exposed to the water scarcely a trace of growth of new tissue appears and no restitution of the missing parts. When, however, two cut surfaces or two parts of a cut surface, *e. g.*, the two margins of a fold at the cut end, come into contact fusion occurs between them, but no further growth takes place unless the region is subjected to tension produced by water in the enteron or in some other way. But when the region is subjected to tension proliferation and growth occur

and a thin membrane of new tissue is formed, whose size is dependent in part on the degree of tension existing. When pieces are cut in such shapes, *e. g.*, by longitudinal or zig-zag cuts, etc., that the cut margins or parts of them do not come into contact with each other, the outgrowth of new tissue does not in any case occur from the free cut surfaces. Such pieces may remain open indefinitely and fail completely to replace the parts removed.

In many cases after the growth of new tissue has begun between approximated parts of the cut surface the new tissue may gradually extend for a very short distance over the cut surface on each side of the point where it began to grow. In such cases it can readily be determined that the thin new tissue is itself under some slight degree of tension. Under these conditions growth proceeds to a certain point and then stops. Apparently the mechanical conditions in the region involved and the physical properties of the new tissue itself are important factors in determining the amount of growth. In my earlier experiments the fact was noted

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that such growth occurs to a much greater extent in *C. membranaceus* where the new tissue is much thicker and more resistant than in *C. solitarius* (Child, '04a, pp. 70–71). In *C. æstuarii*, where the tissues are even more delicate such growth of new tissue does not occur to any great extent. In Fig. 2 the end of a piece cut so that it remains widely open is shown and the extent of the growth of new tissue is indicated by the shaded areas in the angles of the inrolled margins. Such a piece never closes by extension of the new tissue over the whole opening, but remains indefinitely in the condition figured, unless the relation of the parts of the cut margin is altered by changes in the degree of contraction or by passive changes of the position of the piece, which bring new portions of the cut margins into contact or proximity. After such a change growth of new tissue may continue for a time where the angle between two adjoining parts of the cut margin is not too great, but in no case does the new tissue extend with

a free margin across wide spaces as in *C. membranaceus* (Child, '04*a*, Fig. 31).

If the piece becomes distended with water after closure of a cut end by new tissue the new tissue, which at first is scarcely visible between the margins of the old parts, increases in area, but in the absence of distension such growth never occurs. Fig. 3 on p. 277 of my earlier paper (Child, '04*b*) shows the growth of new tissue in a collapsed piece of *C. solitarius* and Fig. 4 the growth in a distended piece. The differences in *C. æstuarii* are similar.

There is then no escape from the conclusion that mechanical strain is a necessary factor in the growth of new tissue in these species. That it is the only factor, I should be the last to assert, but I think it is sufficiently clear that in its absence the *Cerianthus* material possesses no inherent power to restore missing parts.

II. The Control of Tentacle-Development in Regulation.

(a) By Artificial Openings in the Body-Wall.

After the closure by new tissue of the ends of pieces distension of the enteric cavity with water occurs before any opening into the enteron exists. The passage of the water through the body-wall is probably not a simple osmotic process but something more complex. When the mouth is present or after a new mouth is formed water may enter through it, and by these means a certain degree of distension is maintained. In my experiments with *C. solitarius* it was possible to prevent, at least in large measure, this distension by making openings in the body-wall and reopening them at short intervals (Child, '04*c*). Under these conditions the development of the tentacles is retarded or almost entirely inhibited.

The secretion of slime about the opening and the inrolling of the margins of the cut make it impossible to prevent all distension, but by removing the slime and reopening the pieces every few hours or even once a day the distension can be reduced far below the normal and the first appearance and the rapidity of development of the tentacles greatly retarded and their final size much reduced. If it were possible to eliminate all internal pressure the tentacles would undoubtedly fail to appear.

Experiments along this line on *C. æstuarii* confirm my earlier work. It was possible to control the development of the tentacle in this species within wide limits : in some experiments, for example, they attained a length of 1 mm. in the open pieces while in the controls they reached the length of 15 mm. in the same time.

(b) Pieces from the Œsophageal Region.

The œsophagus in *Cerianthus* extends a considerable distance aborally from the mouth (Fig. 1), and pieces in which the œsophagus extends through the whole length can readily be obtained. The process of closure of the cut ends in such pieces was described for *C. solitarius* in my earlier paper (Child, '04d, pp. 205– 206, Figs. 7–15), and is similar in *C. æstuarii*. In almost all cases the cut ends of œsophagus and body-wall unite at both ends of the piece and the œsophagus therefore opens to the exterior at each end but does not communicate with the enteron, at all. In these pieces water can pass into the enteron only through the body-wall, but distension by this means does not continue indefinitely (Child, '04d, pp. 206, 211) and after a few days the pieces gradually collapse and never become distended again. Under these conditions the development of the tentacles begins but ceases as the distension decreases, and later, when the piece becomes completely collapsed, the tentacles undergo atrophy (Child, '04d, pp. 207–212). The results of experiments of this kind on *C. æstuarii* are even more striking, for the atrophy occurs more rapidly. The records for two series of experiments are given by way of illustration.

Series 14.

August 30, 1905. — I. A piece with oral end about the middle of the œsophageal region (*a*, Fig. 3) and aboral end just distal to the aboral end of the œsophagus (*b*, Fig. 3).

II. As control a piece from another animal with oral end as nearly as possible at the same level as that of I., and with aboral end just proximal to the aboral end of the œsophagus (*c*, Fig. 3).

The difference in length of the pieces is very slight and both are far above the minimal size of pieces capable of complete restitution.

September 3. — I. Body-wall and œsophagus united at both

ends; piece somewhat distended with water; marginal tentacles
just beginning to appear, about 1 mm. in length; no labial ten-
tacles (Fig. 4).

II. Body-wall and œsophagus united orally so that a mouth
is formed. Aborally the cut end of the body-wall is closed.
The œsophagus therefore opens into the enteron. The piece is
distended and the marginal tentacles are 2 mm. in length. No
labial tentacles (Fig. 5).

September 9. — I. Piece slightly distended; marginal tentacles
barely visible; no labial tentacles.

II. Fully distended; marginal tentacles 5–7 mm. in length;
labial tentacles just visible.
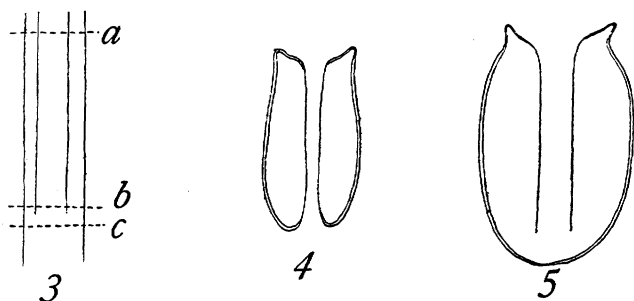
September 18. — I. Collapsed; neither marginal nor labial ten-
tacles visible.

II. Fully distended; marginal tentacles 8–10 mm. in length;
labial tentacles 1–2 mm.

October 8. — I. Collapsed; much reduced in size and body-
wall undergoing atrophy. No traces of tentacles.

II. In burrow, distended; marginal tentacles 18–20 mm.;
labial tentacles 4–5 mm.

Later History. — I. After October 8 complete atrophy of the
body-wall along the line of folds occurred and the piece broke
up into fragments which continued to decrease in size and undergo
atrophy until they died.

II. Remained in good condition in burrow until December 31
when experiment concluded. Marginal tentacles 20–25 mm. in
length; labial tentacles 5 mm.

The difference in the process of regulation between these two
pieces is very great. In piece I. the development of marginal

tentacles began during the period of temporary distention, but after collapse these tentacles atrophied and no trace of labial tentacles ever appeared. Moreover the piece gradually underwent decrease in size and atrophy and finally broke up and died.

Piece II., on the other hand, gave rise to a normal animal with both marginal and labial tentacles and remained in good condition for months.

Series 35.

September 15, 1905. — Four œsophageal pieces from different individuals, each including about the middle half of the œsophageal region.

September 20. — In all the œsophagus and body-wall have united at both ends and the enteron is without communication with the exterior. The pieces are slightly distended and one shows slight elevations of the body-wall in the marginal tentacle region ; the others are without any trace of marginal tentacles, and none show any trace of labial tentacles.

October 2. — All collapsed and reduced in size. No traces of tentacles in any. In three pieces the body-wall has atrophied and split along the line of longitudinal folds.

Later History. — All four pieces gradually atrophied and broke up and the fragments died. No further traces of tentacles appeared at any time. During the same time other pieces of the same and smaller size but with aboral ends proximal to the œsophageal region, closed, became distended, gave rise to marginal and labial tentacles and remained in good condition until the conclusion of the experiment on December 31, 1905.

In general the development of marginal tentacles is almost completely inhibited in œsophageal pieces and the tentacles which do appear undergo complete atrophy later. Labial tentacles have never been seen in the pieces. Moreover the pieces are incapable of continued existence for more than a few weeks in the absence of internal pressure.

(*c*) *The Development of Tentacles on Oblique Oral Ends.*

In *C. solitarius* the formation of tentacles on oblique oral ends is not simultaneous about the whole margin as it is on transverse ends, but the tentacles appear earliest on the most distal portion

of the oblique surface and latest on the most proximal portion
(Child, '04*d*, pp. 193–205, Figs. 1–6). At least a part of this
difference is due to the difference in level of the most distal and
most proximal portions of the oblique surface, but in the paper
referred to it was shown that the marginal tentacles appear earlier
on the most distal portion of an oblique end and later on the most
proximal portion than on transverse ends at these two different
levels. These and other results led me to suggest that perhaps
the currents which pass orally along the inner surface of the body-
wall in each interseptal chamber might, as well as the general in-
ternal pressure, be a factor in tentacle-development (Child, '04*d*,
pp. 193–205).

The results of experiment on oblique pieces in *C. æstuarii* are
similar : part of the records for one series is given here.
Series 33.

September 15, 1905. — I. The oral ends were removed from
four specimens by oblique cuts through the œsophageal region
(*bb*, Fig. 1).

II. The oral ends were removed from four other specimens by
transverse cuts as nearly as possible at the same level as
the most distal portion of the oblique cuts (*aa*, Fig. 1). No
control was made for the lower level of the oblique surface
because the rapidity of tentacle-formation on transverse surfaces
is not very different at these two levels. The history of one of
the oblique pieces and one of the controls is given.

September 17. — The cut ends in the oblique pieces I. and the
control II. were closed : tentacles were not visible in either, but
the body-wall was becoming thinner in the most distal portion of
the oblique piece (Fig. 6, diagrammatic longitudinal section).

September 20. — I. Marginal tentacles about 2 mm. long on
the most distal portion of the oblique surface and decreasing in
length from this point to zero about half way between the
extreme distal and proximal portions of the disc. The most
proximal portion of the disc shows no preparation for tentacle-
formation as yet. Fig. 7 is a diagrammatic longitudinal section
and Fig. 8 shows one side of the disc.

II. Marginal tentacles just appearing about the whole margin
of the disc : 0.5–1 mm. in length (Fig. 9).

September 23. — I. Marginal tentacles on most distal portion 5–6 mm. in length, decreasing to slight elevations on the opposite side (Fig. 10).

II. Marginal tentacles 2–3 mm. in length about the whole margin (Fig. 11).

Later the tentacles on the oblique piece underwent a gradual equalization and the disc lost its obliquity as in oblique pieces of *C. solitarius.*

This series shows very clearly, and my other series afford similar results, that tentacle-formation is accelerated on the more
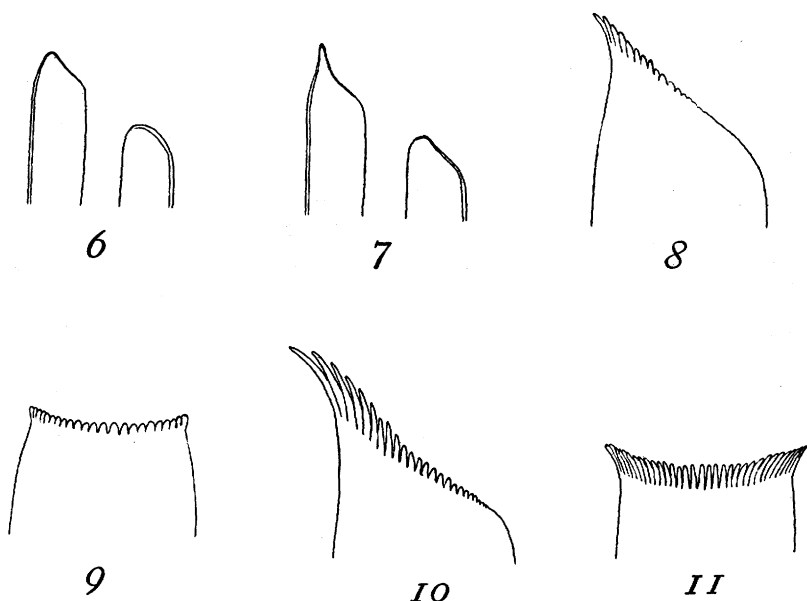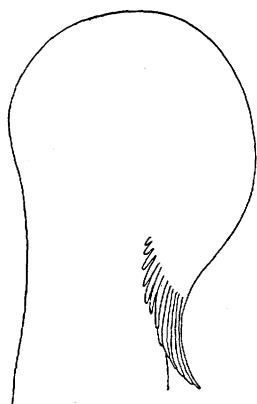

distal portions and retarded on the more proximal portions of the oblique piece as compared with transverse pieces.

Since internal pressure is in general so important a factor in tentacle-formation in *Cerianthus* it seems not at all improbable that the differences between the oblique and transverse pieces may be due to local differences in the internal pressure in the oblique pieces. The currents passing orally in each interseptal chamber enter the inrolled portion of the oral end and, as was suggested in my earlier paper, local differences in the internal pressure may be produced by these currents since the body-wall is always

folded over much more sharply on the more distal portions of
the oblique end than on the more proximal. At all events the
obliquity of the disc affects the rapidity of tentacle-formation in
some manner, and the suggestion that local differences in internal
pressure resulting from the different relation between the internal
currents and the body-wall on different parts of the oblique end
are concerned in producing the result observed seems at present
to account for the facts. The question as to whether localized
internal pressure is a factor in the localization of tentacles will be
considered elsewhere in connection with certain experiments on
another actinian.

One other series of experiments in which the plane of section
of the body was very oblique deserves brief mention. In this
series the plane of section extended from a region near the orig-
inal tentacles on one side to a point con-
siderably below the proximal end of the
œsophagus on the other (*cc*, Fig. 1). The
margin of the body-wall and the œso-
phagus united down to the proximal end
of œsophagus, but since the cut extended
a considerable distance beyond this point,
there remained an opening of consider-
able size, which could be closed only by
approximation of the margins of the
body-wall and the formation of new tissue
between them : this, however, occurred
very slowly. The result of the opera-
tion was then at first a piece in which
one side of the body-wall in the œsophageal region and to a
point some distance proximal to it, and one side of the œsopha-
gus were removed.

The distal portions of these pieces underwent considerable de-
crease in length so that the obliquity of the oral end was reduced.
In earlier stages distension with water was impossible because of
the opening below the œsophagus, and tentacle-formation was
much delayed. Later the most distal portion bent over and to-
gether with the slime secretion effected a provisional closure
(Fig. 12), and some degree of distension took place and was fol-

lowed by the formation of tentacles.   Below the œsophagus no tentacles formed because here no closure of the wound took place.

A month after the operation the pieces possessed the form shown in Fig. 12.   The most distal part of the oral end was bent over so that the oblique disc was applied to the side of the body in the region of the opening below the œsophagus.   The disc bore a semicircle of marginal tentacles which showed the usual differences in length of tentacles.

These pieces are of interest because they show an extreme type of reaction to the wound, but even in this case the reaction seems to consist essentially of contraction of the cut edges of the body-wall.   The fact that this contraction produces so marked a change in the position of the oral end is due merely to the position of the cut.

III. Experimental Change in Length and Atrophy of Tentacles.

The marginal tentacles are 40–50 mm. in length when fully distended and the labial tentacles about 10 mm.   Exact measurements are of course out of the question.   Reduction and atrophy of the tentacles can be induced in this species as in *C. solitarius* by preventing distension of the body and tentacles.   As has been noted above, openings in the body-wall do not entirely prevent distension, even when frequently reopened, for provisional closure occurs, sometimes within a few minutes after reopening.   These provisional closures, however, cannot withstand anything like the normal internal pressure, so that even when they occur the total distension is very much below the normal.

In one series of this kind the tentacles were reduced in fifty days from full length to mere stumps, the marginal tentacles being 2 mm. in length and the labials just visible as slight elevations.   Atrophy of the tentacles proceeds in the centripetal direction as in *C. solitarius*, and at the tip of each tentacle a small mass of degenerating tissue is visible (cf. Child, '04*e*, Figs. 3–5).   If the specimens are allowed to close and become distended after atrophy of the tentacles, growth of the tentacles begins anew and proceeds according to the degree of distension established.

Tentacle-atrophy is a characteristic feature of pieces taken

entirely from the œsòphageal region, in which the œsophagus and
body-wall unite at both ends and there is no communication be-
tween the enteron and the exterior. The distension which occurs
during the first few days after closure disappears later and com-
plete collapse takes place. Under these conditions atrophy of the
tentacles is very rapid : in some cases complete disappearance
of fully developed normal tentacles occurred within thirty days.
Tentacle-atrophy is always followed in these pieces by atrophy
of the body-wall and finally fragmentation and death of the
pieces.

Partial tentacle-atrophy can be induced in this species merely
by keeping the animals in dishes without sand in which they can
burrow. Without the support of the wall of the burrow the
body-wall is unable to sustain the normal degree of internal
pressure and the tentacles undergo partial atrophy in conse-
quence of the altered conditions. Apparently the internal pres-
sure is regulated to a greater or less extent by the strain upon
the body-wall, for it is certain that in animals outside the bur-
rows the internal pressure is not nearly as great as when the
body-wall is supported by the wall of the burrow. So far as I can
determine this difference is not due merely to escape of the water
through the aboral pore when a certain degree of pressure is
reached, for the pressure is always much below the point when
opening of the aboral pore occurs : there seems rather to be a
regulation of the entrance of water. Occasionally for some
reason regulation does not occur or is insufficient and rupture
of the body-wall takes place, usually in the aboral third, where
the body-wall is thinner than in other regions.

After animals have been kept for five or six weeks under these
conditions the marginal tentacles are usually 10–15 mm. and the
labials about 5 mm. in length.

A marked difference in the length of tentacles produced in
regulation likewise appears according as the animals are allowed
to burrow or are kept without sand. Tentacles developing on
pieces which live in burrows may attain the length of the origi-
nal tentacles while those on pieces without sand reach only half
this length or less.

The body-wall of *C. solitarius* is much thicker and more resist-

ant than that of *C. æstuarii* and is capable of supporting without the aid of the tube, a degree of internal pressure which closely approaches or perhaps equals the normal. In that species, therefore no marked reduction of the tentacles is observed in specimens kept without sand.

IV. LATERAL PARTIAL DISCS.

The formation of lateral partial discs occurs in connection with lateral incisions and usually in the œsophageal region only, where the cut edges of the body-wall and the œsophagus fuse both above and below the cut and so give rise to a lateral mouth (Child, '05*a*). In all cases observed in *C. solitarius* the region between the lateral cut and the oral end of the body undergoes complete atrophy sooner or later and the lateral partial disc takes the place of the atrophied part. Atrophy occurs in this region because its enteric cavity has no connection either with the other portions of the enteron or with the exterior. After closure some distension occurs but collapse follows and continues until the part undergoes complete atrophy. Meanwhile the partial disc gradually changes its position toward the oral end and finally replaces the atrophied part.

#### (*a*) *Transverse Lateral Incisions.*

The only difference worthy of note in this connection between *C. solitarius* and *C. æstuarii* is the more rapid atrophy of the portion oral to the cut in the latter species. Frequently this atrophy is so rapid that breaks occur in the piece within a week or ten days after the operation, while in *C. solitarius* this condition is reached only after several weeks. Fig. 14 shows a longitudinal section of a specimen with lateral partial disc and the collapsed portion undergoing atrophy oral to it. The incision in this case was made at *a*, Fig. 13.

(*b*) *Oblique Lateral Incisions Directed Aborally.*

No oblique lateral incisions were made in my earlier work on *C. solitarius*, but a considerable number of operations of this kind have been made upon *C. æstuarii*. When the incision is directed aborally at any angle with the transverse plane up to 50°–60° (*b*, Fig. 13) partial discs appear which show the difference in

rapidity of tentacle-development of different parts of the disc characteristic of oblique discs (cf. pp. 35–38), the tentacles appearing earliest on the most distal portion and latest on the most proximal portion of the disc. A case of this sort is shown in Fig. 15. The later history of these oblique discs is similar to that of partial discs formed after transverse incisions, and during the change in position of the disc the obliquity gradually disappears as in other oblique discs and the result is an animal of usual shape.

But when the angle between the incision and the transverse plane is greater than 50°–60° (*c*, Fig. 13) the results differ from
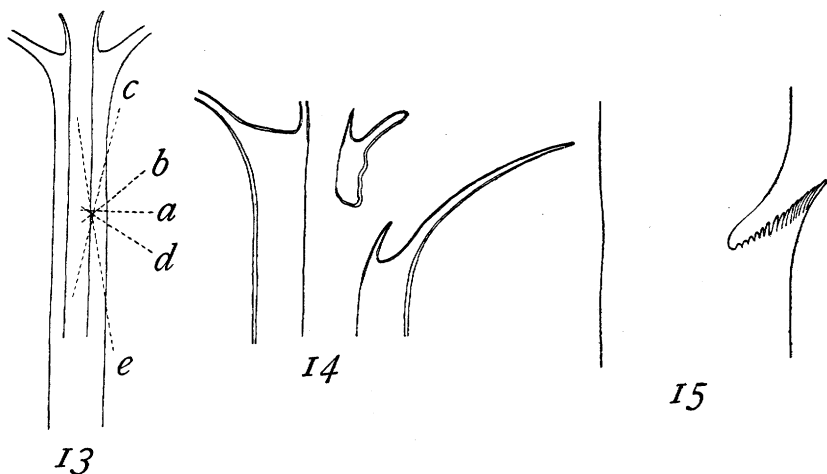

those described and in some cases permanent lateral discs are formed borne on a small column which arises as a branch from the side of the original body. As the plane of the incision approaches the longitudinal axis of the body the incision approaches a longitudinal direction and the process of wound-closure approaches that occurring after a longitudinal incision. The course of an oblique incision approaching the longitudinal direction is indicated in Fig. 16. After an incision of this kind the wound in the body-wall closes longitudinally by approximation and union of the cut margins (Fig. 17), but the oblique slip of tissue which was separated distally from the body-wall but is still connected with it proximally is not included in the closure and its cut margins also close longitudinally and independently of the

other part except at its proximal end where it is continuous with the rest of the body-wall. Thus the closure of the wound results in the formation of a small component extending obliquely in the distal direction from the side of the body. The œsophagus also closes longitudinally in each part and in the lateral component unites distally with the body-wall so that this component possesses a mouth. In this manner an oblique lateral disc is established at the distal end of the lateral component, but it is a complete not a partial disc, because the closure has been largely longitudinal. On such discs the tentacles arise in the manner characteristic for oblique discs, *i. e.*, earliest on the most distal portion and latest on the proximal portion of the disc (Fig. 18).

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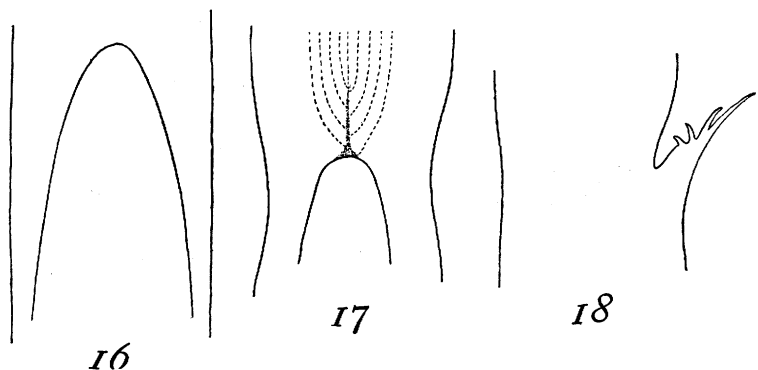
In Fig. 19 one of these cases is shown after complete development of the tentacles on the lateral disc, but before the obliquity has disappeared. In this figure it is seen that several of the tentacles on the original terminal disc, viz., some of those which were situated directly distal to the incision, have undergone partial atrophy. The stage figured does not, however, represent the greatest degree of atrophy which occurred. After the closure of the wound these tentacles underwent rapid atrophy until they were reduced to less than half the length shown in the figure and some of them were mere stumps 2–3 mm. in length. Then they began to grow again and would undoubtedly have finally attained the same length as the others if it had been possible to continue observation of the specimen for a longer time. The reason for the peculiar history of these tentacles is to be found in the method of closure of the wound. In Fig. 17 the direc-

tion of the septa in the region of the incision is indicated dia-
gramatically by the dotted lines. With the approximation of the
oblique margins of the cut the cut ends of the septa are also
closely approximated, and since the œsophagus closes in the
same manner as the body-wall the consequence is that some of
the interseptal chambers distal to the apical region of the cut be-
come almost or completely closed aborally, while lower down,
where more new tissue is formed between the old margins of the
body-wall they remain more widely open. When the body is
distended with water those chambers which are nearly or quite
closed aborally remain almost or wholly collapsed, since they
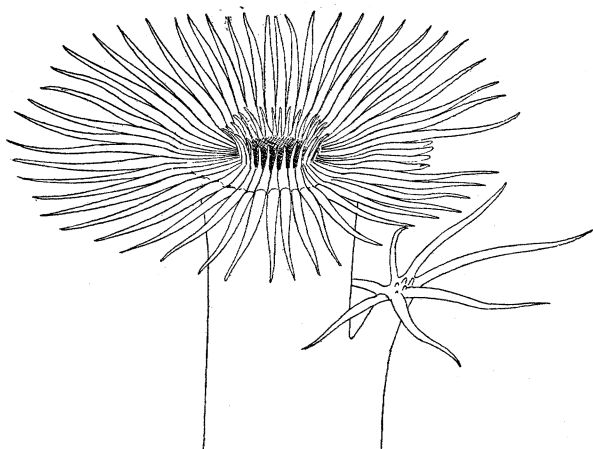
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are nearly or quite isolated from the other parts of the enteron,
and the pressure resulting from the more rapid distension of other
parts aids still further in closing small openings which may re-
main. After the distension resulting from the passage of water
through the body-wall or septa has subsided this region remains
collapsed and the tentacles corresponding to those chambers
begin to atrophy. But in the further course of regulation after
closure the area of new tissue increases and the old margins of
the cut, now united by a thin membrane of new tissue, gradually
separate as it increases, and the relations of the septa also un-
dergo regulation in consequence. At a certain stage the inter-
septal chambers which were shut off from other parts of the

enteric cavity again become open and fill with water and renewed distension of the tentacles and consequently renewed growth occurs.

But the most interesting feature of these cases is that, so far as my observations go, the lateral component is permanent and does not undergo further regulation ending in union with the parts of the terminal disc which remain, as do the transverse and less oblique lateral discs.   Some specimens were kept under observation during four months and at the end of this time there was no indication of any further regulation leading to fusion of the lateral component with the rest of the body.   Fig. 19 is drawn from one of these specimens four months after the operation.   This length of time is more than sufficient for the migration of the lateral disc to the oral end of the whole if it were to occur, but there is no evidence of any such change in position.   Each component maintains its form and relations to the other.   The only changes noted in later stages were, the increase in length of the lateral component, which would probably in time have reached the level of the original disc, the gradual reduction of the obliquity of the disc of the lateral component and new growth of the previously atrophied old tentacles above the cut.

The reason for the failure of the lateral disc to migrate to the oral end of the body and take the place of a part of the original disc lies in the simple fact that no part of the region distal to the cut underwent complete atrophy.   Atrophy began, but the regulatory process involved in the filling of the area of the cut with new tissue brought about the opening and renewed distension of the interseptal chambers in this region and so determined its persistence.   Evidently the presence of active non-atrophied parts distal to the lateral component prevents its migration to the oral end.

These cases are of considerable interest in that they indicate very clearly the dependence of "normal" proportions and positions of parts upon certain factors which are subject to experimental control, viz., in this case the direction of the incision and the distension or absence of distension of certain parts with water and their consequent persistence or atrophy.

(c) *Oblique Lateral Incisions Directea Orauy.*

When the oblique incision is directed orally the results differ, according to the angle as in the case of aborally directed incisions described in the preceding section. If the angle between the incision and the transverse plane is not greater than 30–40 (*d,* Fig. 13) a lateral partial disc with mouth is usually formed, the parts distal to it atrophy and it gradually migrates to the oral end of the body. The sequence of tentacle-formation in these partial discs is that characteristic of other oblique discs, the tentacles appearing earliest on the two opposite ends of the partial disc, which in these cases are the most distal portions, and latest on the middle, most proximal portion.

When the orally directed incision is more oblique, however (*e,* Fig. 13) other results are obtained. In most cases of this sort complete closure of the wound occurred sooner or later without the formation of a lateral mouth or partial disc, *i. e.,* the cut margins of the body-wall united longitudinally with each other and not with the œsophagus, and the oblique slip, which in these cases is directed aborally, formed a small shrivelled excrescence, which underwent gradual atrophy and resorption.

After these incisions the margins of the body-wall approximate and unite longitudinally from the proximal end of the cut distally (Fig. 20, the area of new tissue is indicated by shading). When the approximation of the margins and the accumulation of slime has brought about a provisional closure and partial distension is possible, the more distal portions of the cut margin are pressed against the aborally directed slip which overlaps this region and union readily occurs here, so that the whole wound is closed without the formation of a disc. Here the result, in itself different from that obtained with aborally directed oblique incisions, is brought about by the same internal factors, *i. e.,* the characteristic reactions of the species, but the conditions under which the reactions occur are different, hence the difference in result. In the case of aborally directed incisions the partial distension separates the oblique slip from the rest of the body-wall, since the slip shares more fully in the distension than the portion of the body-wall directly distal to the cut. In the present case, on the other hand, the oblique slip is less distended than other

parts, hence it contracts and the other parts are pressed against it, permitting union of the cut margins to occur.

In several cases of this sort the incisions were reopened one or more times after they had healed in the manner above described, and in one case tentacles were produced, but later disappeared. The history of this case is briefly given since it presents some features of importance.

Series 31—III.

September 1. — Very oblique incision directed orally.

September 9. — Almost entirely healed ; reopened.

Marginal tentacles on original disc 12–15 mm. in length except

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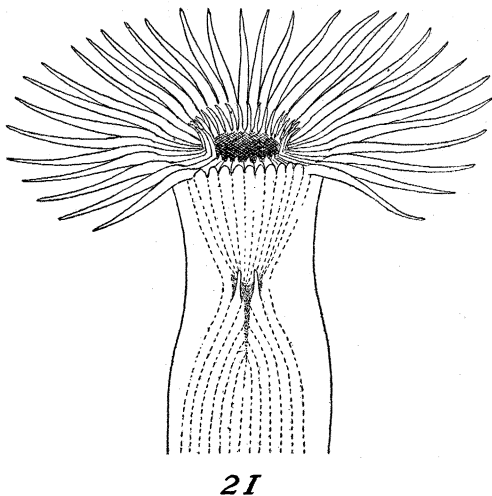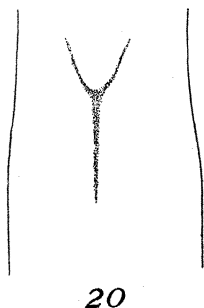
above cut, where they are reduced to about 1 mm.   Labial tentacles 3–4 mm., except over cut, where 1–2 mm.

September 28. — (Fig. 21.) Marginal tentacles on original disc 10–12 mm. in length except over cut, where they are reduced to about 1 mm.   Labial tentacles 2–3 mm., except over cut, where they are scarcely visible.

The lateral wound is closed by thin new tissue, but near its distal end two small tentacles appear, one arising from each side of the cut.   Proximal to the tentacles the cut margins have united longitudinally and distal to the tentacles a broader area of new tissue fills in the space between the old margins.   The course of the septa is indicated by the dotted lines in Fig. 21.   It is evident

that tentacles could not arise proximal to the two which have ap-
peared, because there the two sides of the cut united almost
directly.   The two tentacles represent the last interseptal chamber
on each side which was opened by the incision.   The next inter-
septal chambers lateral to these on each side are continuous to
the oral end of the body and the first of the normal tentacles on
each side above the cut corresponds to one of them.   The reason
for the atrophy of the old tentacles above the cut is at once ap-
parent from the figure.   In consequence of the approximation of
the sides of the cut and the contraction of the oblique slip the
interseptal chambers to which these tentacles belong have been
cut off from communication with the rest of the enteron.   In the
living specimen this portion of the body was very evidently col-
lapsed, while other parts were distended.

   October 11. — No marked changes since September 28.

   December 31. — During the period between October 11 and this
date the specimen was not examined closely since the formation
of tentacles in the lateral region was the chief purpose of the
experiment.   On this date, when the experiment was concluded,
examination showed that the two small tentacles had disappeared,
the new tissue which closed the wound had become thicker and
more like the old body-wall and extended over a larger area than
before, and finally the tentacles above the cut on the terminal
disc had once more grown out to the same length as the others.
At this time the marginal tentacles were 7–8 mm. in length and
the labials 1–2 mm.   In the course of regulation the contracted
area resulting from the approximation of the margins of the cut
had again spread out in consequence of the growth of new tissue
and the interseptal chambers were again in communication with
the rest of the enteron, hence the tentacles corresponding to them,
which were previously atrophied, had developed to the same
length as the others.

   During a considerable part of this time the animal was in a
burrow which it had made for itself in the sand, and it is probable
that the pressure of the body-wall against the wall of the burrow
aided in bringing about the disappearance of the two tentacles.

   In this case, as in those discussed above, the special result is
determined by the conditions of the experiment.   No new method

of regulation is involved, but merely the same complex of reactions as in other cases under different conditions.

From these experiments with transverse and oblique lateral incisions it is evident that the formation of a lateral partial disc (Figs. 14 and 15), a lateral complete disc borne on a column arising like a lateral branch from the side of the body (Fig. 19), the formation of a few tentacles which disappear later (Fig. 21), or the closure of the wound without formation of a disc may each be determined by the conditions of the experiment.   These different results cannot be interpreted as in any sense purposive or adaptive responses of the animal to the special conditions, since the nature of the reactions themselves is not altered, but merely their position, relation and sequence, and these are very clearly determined by the conditions of the experiment.

The Effect of Internal Pressure and its Absence upon the Body-Wall in General.

As was noted above, the body-wall of this species is thinner and more delicate than that of *C. solitarius* and very much more so than that of *C. membranaceus*.   It is also much more sensitive and reacts more rapidly to the mechanical conditions resulting from internal pressure than do the tissues of either of those species.

Pieces of *C. solitarius* cut in such manner that closure is impossible remain alive for months, though they undergo gradual decrease in size and atrophy and finally break up and disintegrate. Pieces of *C. æstuarii*, on the other hand, in which no distension is possible, atrophy much more rapidly.   The atrophy begins at points where the body-wall is sharply folded, and splits and breaks often occur in such regions within a week or ten days after the operation.   The whole piece often breaks up and disintegrates completely in the course of three or four weeks — sometimes even more rapidly.   Particular regions of the body-wall which remain collapsed for any considerable length of time undergo complete degeneration and disintegration.   In short, it is evident that an essential condition, not only for the formation of new substance, but for the continued existence of the body-wall and tentacles in this species is the mechanical condition resulting from distension of the enteron with fluid.

On the other hand, the body-wall of this species is incapable
of supporting alone the pressure of the enteric fluid.   Under
normal conditions the body-wall is supported by the wall of the
burrow in which the animal lives and so does not support the
entire pressure.   When the animals are kept without sand in

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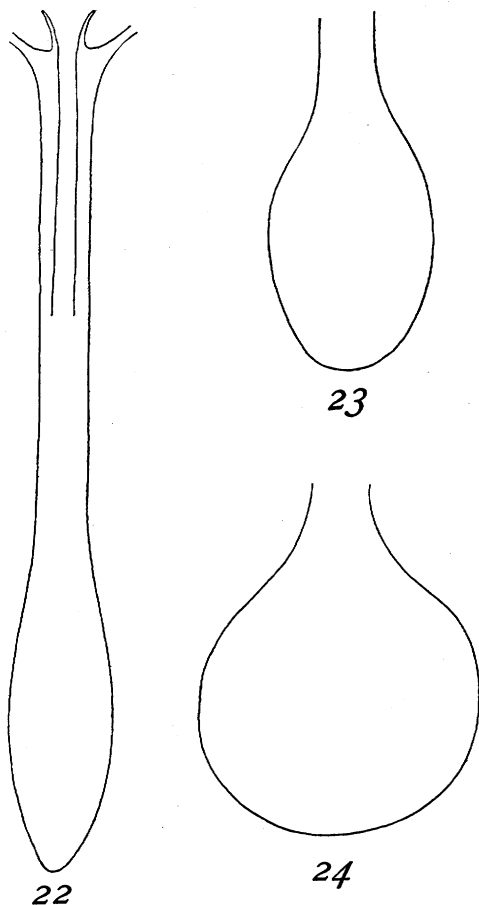
which to burrow the shape of the body undergoes marked changes,
especially at the aboral end, where the wall is thinnest.   Under
these conditions the aboral region of the body gradually increases
in diameter and decreases in length (Fig. 22) and the change
involves more and more of the body (Fig. 23), until in some cases
almost the whole body aboral to the œsophagus approaches a

spherical form (Fig. 24). Such specimens are absolutely incapable of burrowing and never regain their usual form, at least no return was observed during a period of something over four months. Frequently, however, the change in shape ceases before the extreme condition is reached, *e. g.*, at a stage resembling Fig. 23 and the animal retains this shape afterward. The cessation of the change in shape appears to be a functional reaction of the tissues to the altered conditions to which they are subjected, *i. e.*, the increased tension in the body-wall develops increased resisting power, a change similar to that occurring in many other so-called functional adaptations. On the other hand, rupture of the body-wall frequently occurs in the enlarged region. These facts demonstrate that the tubicolous habit is an important factor in determining the shape of the body and the functional character of the body-wall.

VI. DISCUSSION AND SUMMARY.

C. æstuarii demonstrates even more clearly than *C. solitarius* the importance of internal water-pressure for regulatory development and for the continued existence of the body-wall and tentacles.

On the other hand, there is no evidence that changes in the turgor of the cells themselves play an important or definite rôle in form-regulation, except that atrophy appears to be accompanied by a decrease in the turgor of the tissues involved. There is certainly no appreciable change in the turgor of the cell such as Moszkowski ('07, p. 412) describes for *Actinia* and *Actinoloba*. If Moszkowski's observations are correct, and certainly observations on *Cerianthus* do not permit conclusions concerning other forms, they simply afford another illustration of the physiological differences which may exist in different species.

The passage of water through the body-wall and the consequent distension of the body occurs in *Cerianthus*, as noted above and in my earlier papers (Child, '04*b*, pp. 276–277), as well as in *Actinia* and *Actinoloba* (Moszkowski, '07, p. 412), but it has not been possible thus far to discover any indications of a marked change in turgor of the cells themselves, and the results of my experiments show very clearly that the water in the enteron is an essential factor in form-regulation in *Cerianthus*.

According to Moszkowski ('07, p. 420), the decreasing rapidity of regulation with increasing distance from the oral end of the body is due in *Actinia æquina* merely to the fact that below a certain level a new œsophagus is formed and the parts of the old œsophagus which remain hinder closure until they are cast off.

In *Cerianthus* this is not the case. In all of the observed cases any portion of the œsophagus which remains takes part in the process of regulation and is not cast off. Moreover, differences in the rapidity of regulation are distinguishable both at different levels within the œsophageal region and at different levels aboral to it. It is to be expected that any difference in capacity which might exist at different levels of the body would be more marked in the greatly elongated body of *Cerianthus* than in such a form as *Actinia*, where the body is relatively short. At any rate, there is no doubt that such a difference exists in very considerable degree in *Cerianthus*.

The most important results of my experiments with *C. æstuarii* are stated in the following summary.

1. In *C. æstuarii*, as in *C. solitarius*, the distension by water in the enteric cavity is an essential factor in form-regulation. In its partial or total absence the formation of disc and tentacles is retarded or inhibited.

2. The internal pressure is essential not only for the formation of new parts, but for the persistence of the old. Partial or total atrophy of the tentacles follows decrease or absence of distension and the atrophied structures develop anew when distension is again permitted to occur.

3. The body-wall of *C. æstuarii* is much thinner and more delicate than that of *C. solitarius* and is also much more sensitive to changes in internal pressure. In the absence of distension even the body-wall undergoes rapid atrophy and disintegration.

4. In nature the walls of the burrow in which the animal lives aid the body-wall in supporting the pressure resulting from distension, especially in the aboral region. If the animals are kept in water without sand in which to burrow, the internal pressure never reaches its normal amount. Under these conditions the tentacles are more or less relaxed and undergo partial atrophy and the aboral region of the body becomes greatly deformed and

often ruptures because of its inability to sustain the existing pressure. In some specimens a "functional adaptation" to the altered conditions occurs and the body-wall gradually acquires the strength necessary to support the pressure. In such cases the partially atrophied tentacles may increase in length but in no case observed did they attain the length of tentacles of specimens living in burrows. Regulatory tentacles likewise fail to attain full length when the specimens are kept without sand, but do attain full length when they are permitted to burrow.

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April, 1908.